INTRODUCTION

Due to alarming increase in resistance to antibiotics, the need of the hour is to develop new therapeutic compounds, especially in India owing to the increasing pace of development of antibiotic resistant bacteria pathogenic strains. *Staphylococcus aureus* is one of the prominent bacterial pathogen and its potential to cause wide spectrum of pyogenic lesions involving several organs; hospital outbreaks and community acquired infections are well recognized. *Staphylococcus aureus* infections are associated with resistance to several beta lactum antibiotics used in hospitals. These strains are known as MRSA (Methicillin resistant *Staphylococcus aureus*) [1].

Many recent reports have noted increased prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) as a common multidrug resistant bacteria pathogen [2,3,4]. MRSA is now endemic in India. The incidence of MRSA varies according to the region, 25% in western part of India [5] to 50% in South India [6]. Chemicals obtained from medicinal plants are known as phytochemicals serve as lead potential compounds in drug discovery and design [7]. Plant derived drugs remains important resource especially in developing countries to combat serious diseases. [8]

*Aegle marmelos* belongs to family Rutaceae, commonly known as Bael tree [9], and has been used by the inhabitants of Indian subcontinent for over 5000 years. It’s a slow growing, medium sized tree, up to 12-14 m tall with short trunk, thick bark, alternate leaves, borne singly or in group, composed of 3 to 5 oval, pointed leaflets [10]. Leaves, fruits, stem and roots of the tree at all stages of maturity are used as ethno medicines against various human diseases [11]. In traditional system of medicine, leaves are used as an astringent, laxative, digestive and febrifuge when fresh. They are also useful in ophthalmia, hearing loss and inflammation [12]. Leaves are also used as a great medicine for diabetes, diarrhea, detoxification, skin ailments, asthma, cold and fever [13]. *Aegle marmelos* fruit is traditionally used to treat jaundice, chronic diarrhea, dysentery, fever, asthma, inflammations, ulcers and swelling [14, 15].

Various crude extracts of the plant have shown antibacterial, antiulcer, antioxidant, anti-inflammatory, anticancer, antipyretic effects [16]. *Aegle marmelos* has diverse pharmacological profile due to the presence of wide spectrum range of chemical entity [17].

Based on the background knowledge of therapeutic potential of the plant species and limited work on the antibacterial activity particularly against in particular Methicillin resistant *Staphylococcus aureus* (MRSA), and considering the urgent need to develop alternative therapeutic options, the present investigation was focused to assess the invitro antibacterial effect of *Aegle marmelos* leaves and fruits against MRSA followed by qualitative screening of phytochemicals in the plant extracts responsible for antibacterial activity.

MATERIALS AND METHODS

Plant collection and sample preparation

*Aegle marmelos* leaves and ripe fruits were collected from Thiruvallur District in Tamilnadu. The plant materials were washed thoroughly, shade dried for about 15 days and grounded into a powder. About 25g of plant powder was extracted with 100 ml solvents by using Soxhlet apparatus. Solvents used for extraction were ethanol, chloroform, hexane and water. The resultant extracts were filtered by using Whatman No 1 filter paper and then concentrated in a rotary evaporator and were stored in a refrigerator at 4°C in small sterile glass bottles for further analysis.

Antibacterial activity

The dried plant extracts were dissolved in Dimethyl sulfoxide (DMSO) separately at the concentration of 1mg/ml for antibacterial assay. The bacterial culture used in the study was pure clinical isolates of Methicillin resistant *Staphylococcus aureus* procured from Private Hospital, Chennai. Muller Hinton Agar (MHA) medium was used to study antibacterial activity. Prior to antibacterial screening, the bacterial culture was cultured in Muller Hinton Broth for about 4 hrs at 37°C. Antibacterial testing was carried out by Kirby Bauer disc diffusion method (Bauer et al., 1966.) The bacterial culture was inoculated as lawn culture using sterile swab over the agar surface. The filter paper discs impregnated with 100 microl of plant extract (1mg/ml) were placed on the seeded agar plates. Dimethyl sulfoxide (DMSO) served as negative control and Streptomycin (10 microg) as reference. The plates were then labeled and incubated at 37°C for 24 hours. After incubation, the plates were examined for clear inhibition zone and zone diameters were measured and recorded.

Statistical analysis

The antibacterial assay was done in triplicates. Statistical analysis was conducted using Winks software and p values were computed.

Phytochemical screening

The plant extracts were subjected to preliminary qualitative phytochemical screening method as described by Harbourne [18].
The plant extracts were investigated for tannins, alkaloids, flavanoids, and saponins.

The test of tannins was carried out by boiling 0.5g of sample in 20 ml distilled water followed by addition of 3 drops of 5% ferric chloride to the filtrate. Development of dark green colouration indicated positive by the presence of tannins. The flavanoids was determined using 0.2 g of plant extract was dissolved in dilute sodium hydroxide and adding drops of dilute hydrochloric acid. The development of yellow colouration was taken positive for flavanoids. The test for alkaloids was carried out by treating 1 g of plant with 5 ml methanol and 5ml of 2N HCl and then the filtrate was treated with Mayer’s reagent. Development of precipitate indicated the presence of alkaloids. Saponins were detected by boiling 1 g of the sample with 10 ml distilled water for 15 minutes and the cooled extract was shaken for froth formation.

**Results and discussion:**

In the present study, both *Aegle marmelos* leaf and fruit extracts showed significant antibacterial activity visibly observed by the zones of inhibition. (Fig1). The extracts exhibited bacterial inhibition activity which was solvent dependent effect.

**Table 1: Comparative Antibacterial profile of *Aegle marmelos* Leaf & Fruit extracts on Methicillin resistant *Staphylococcus aureus* (MRSA)**

<table>
<thead>
<tr>
<th>Concentration of the Plant Extract</th>
<th>SOLVENTS</th>
<th>POSITIVE CONTROL (Streptomycin 10 Micro gram)</th>
<th>Aegle marmelos Leaves on MRSA ZOI(mm)</th>
<th>Aegle marmelos Fruits on MRSA ZOI (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mg/ml</td>
<td>Ethanol</td>
<td>18</td>
<td>31 30 30 32 30 32</td>
<td>1 2 3 1 2 3</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td></td>
<td>20 18 20 22 20 20</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td></td>
<td>-- -- -- -- -- --</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
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</tbody>
</table>

No Inhibition: ---; ZOI – Zone of inhibition diameter.

**Fig 1: Zone of Inhibition results of antibacterial activity.**

The results revealed the significant inhibitory effects of *Aegle marmelos* crude ethanolic and chloroform leaf extracts against the bacterial pathogen, Methicillin resistant *Staphylococcus aureus* (MRSA). Among the four extracts tested, ethanolic leaf and fruit extracts exhibited maximum antibacterial activity with the inhibition zone of 31mm and 32 mm respectively compared to the standard drug Streptomycin (positive control) whose inhibitory effect was relatively less (18mm). Chloroform leaf extract showed moderately considerable inhibitory activity with the inhibition zone of 20mm. Hexane and aqueous extracts did not show any activity in both leaves and fruit extracts.(fig 1).

Our results are consistent with previous researchers. According to (Poongothai et al, 2008), [19], significant antibacterial activity was observed with *Aegle marmelos* methanol extracts against *Staphylococcus aureus*. Kothari et al (2011) [20] have reported methanol and chloroform extracts showed variable broad spectrum antibacterial activities. Amit Pandey et al (2011) [21] reported ethanolic and ethyl acetate extracts of *Aegle marmelos* fruits showing the best result while methanolic and hot water showed minimum inhibition. Sudha Maheshwari et al 2007 [22] reported petroleum ether extracts of *Aegle marmelos* as effective solvent showing antibacterial activity while chloroform extracts of the plant demonstrated no antibacterial activity. Comparable to our results, Rama Dhahiya et al (2015) also reported no antibacterial activity in aqueous extracts of *Aegle marmelos* fruits.

Our results indicate ethanolic leaf and fruit extracts of *Aegle marmelos* contain therapeutically useful compounds against MRSA infections. It is likely that amount of active compounds in hexane and aqueous extracts may not occur in quantities large enough to exhibit significant antibacterial activity. Preliminary phytochemical screening analysis showed the presence of tannins, flavanoids, saponins and alkaloids in both ethanolic leaf and fruit extracts of *Aegle marmelos*. The data is shown in Table 2. The antibacterial activity of the ethanolic extracts may be due to the presence of these active phytochemicals. The previous researchers have reported the presence of active phytochemicals responsible for antibacterial activity. Tannins are known to form irreversible complexes with protein and inhibit cell wall synthesis of the bacteria [23]. Previous reports have shown iron binding capacity of tannic acids responsible for bringing about inhibitory bacterial activity [24].

**Table 2: Qualitative Phytochemicals screening of *Aegle marmelos* ethanolic leaf and fruit extract.**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Phytochemicals</th>
<th>Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Flavanoids</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
</tbody>
</table>

**CONCLUSION**

From the results obtained, Our study highlights the significant antibacterial activity potential of ethanolic leaf and fruit extracts of *Aegle marmelos* against Methicillin resistant *Staphylococcus*
This study serves as a preliminary scientific validation of *Aegle marmelos* Ethanolic extracts as an important source for development of therapeutic antibacterial compounds against MRSA Infections. However the exact structure of chemical components and mode of action of phytochemicals are currently not clear. Our next line of further investigations is a fractionation, purification and characterization of the bio active components in the ethanolic leaf and fruit extracts of *Aegle marmelos*.

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REFERENCES